J Korean Soc Appl Biol Chem (2015) 58(1):35–43
DOI 10.1007/s13765-015-0048-4

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Long-term fate of the herbicide mefenacet in a rice-grown lysimeter over a period of 6 consecutive years

Kee Sung Kyung · Ki Chang Ahn · Jeong Wook Kwon · Yong Pil Lee · Eun Young Lee · Yun Jeong Kim · Fritz Führ · Jae Koo Lee

Received: 3 October 2014 / Accepted: 4 November 2014 / Published online: 11 February 2015

Abstract To show the long-term fate of the herbicide mefenacet in rice paddies, [aniline-^{14}C]mefenacet was applied to a lysimeter of loamy soil with a depth of 1 m, and rice plants were cultivated for 6 consecutive years according to the conventional methods in Korea. Mineralization of [^{14}C]mefenacet to $^{14}CO_2$ and volatilization from the soil surface were 12.01 and 0.02 %, respectively, of the originally applied amount during the first 23 weeks following application. Throughout the 6-year period, the total proportion of ^{14}C-radioactivity that leached through the lysimeter soil was 0.778 % of the original radioactivity. The total ^{14}C-radioactivity absorbed and translocated by rice plants throughout the 6 years was 2.46 % of the applied ^{14}C. Measurement of the ^{14}C-radioactivity distributed in each part of the rice plant (the straw, the ears without rice grain, the chaff, and the brown rice grain) indicated that the amount of ^{14}C in straw was 25.87 times higher than that in brown rice grain over the 6 years. The ^{14}C-radioactivity remaining in the soil layer after 6 years was 44.58 % of that applied, 91.45 % of which was distributed in the upper 0–20-cm layer. These results strongly indicate that mefenacet moved downward very slowly and more than half of the herbicide applied was released into the air, mainly through mineralization to CO_2 in soil during the experimental period.

Keywords Herbicide · Long-term fate · Lysimeter · Mefenacet · ^{14}C-radioactivity

Introduction

Mefenacet (2-(1,3-benzothiazol-2-yloxy)-*N*-methylacetanilide) introduced by Schmidt et al. (1984) is a selective herbicide used as pre-emergence and early post-emergence

K. S. Kyung (✉) · J. K. Lee
Department of Environmental & Biological Chemistry, Chungbuk National University, Cheongju 361-763, Korea
e-mail: kskyung@cbnu.ac.kr

J. K. Lee
e-mail: jklee@cbnu.ac.kr

K. C. Ahn
Metabolism Group, PTRL West, Hercules, CA 94547, USA
e-mail: kahn@ptrlwest.com

J. W. Kwon
Mississippi State Chemical Laboratory, Mississippi State University, Starkville, MS 39762, USA
e-mail: jkwon@mscl.msstate.edu

Y. P. Lee
Quality Control Team, Young Il Pharm Co., Ltd., Jinchun 365-805, Korea
e-mail: lee8772@hotmail.com

E. Y. Lee
Registration and Regulatory Affairs Team, Dongbu Farm Hannong Co., Ltd., Seoul 135-523, Korea
e-mail: eylee@dongbu.com

Y. J. Kim
R&D Team, Greenhigh Co., Ltd., Cheongju 363-814, Korea
e-mail: lobing-hwa@hanmail.net

F. Führ
Institute of Chemistry and Dynamics of the Geosphere IV, Agrosphere, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany
e-mail: fritz.fuehr@t-online.de

herbicide for transplanted rice in Korea. It has been reported to be effective on annual monocotyledonous and some dicotyledonous weeds in rice paddies. Fedtke (1991) and Hess et al. (1990) reported that the herbicide inhibited cell division and cell enlargement in green alga and oat. Morphological studies on *Echinochloa* spp. treated with mefenacet have been conducted (Ito et al. 1989), and an investigation on the morphological and anatomical effects on barnyard grass was published by Ito et al. (2000). Krauskopf et al. (1989) reported uptake and translocation studies of mefenacet in rice and *Echinochloa crus-galli* in pots for a short period of time under greenhouse conditions. Führ and Mittelstadt (1982) and Lee et al. (1989) reported a substantial difference in uptake and translocation of mefenacet between micro-ecosystem (pot) and lysimeter experiments; in the pot experiments, uptake was about 50 (Führ and Mittelstadt 1982) and 17 (Lee et al. 1989) times higher than in the lysimeter experiment. They attributed differences in the homogeneity of pesticides in soil, the ratio of roots to soil, the supplemental period of water, and climatic conditions at the experimental sites (Führ and Mittelstadt 1982). For these reasons, it is essential to use outdoor lysimeters with undisturbed soil profiles, and radio-labeled compounds to more accurately show the fate of pesticides applied to agricultural fields.

Many researchers have reported that outdoor lysimeters with undisturbed soil structure reflect field conditions (Scheunert et al. 1983; Kubiak et al. 1988; Stork et al. 1994; Schroll et al. 2008). Also, the use of radio-labeled chemicals can significantly increase the accuracy of research on the fate and behavior of polycyclic aromatic hydrocarbons (PAHs) by establishing a mass balance (Abbott et al. 1992). As mentioned above, the use of lysimeters, which can be used to simulate paddy fields with radiotracers, is an ideal technique for the study of fate of pesticides, including the study of uptake and translocation by crops, metabolism in soil–crop–water systems, movement and leaching in the soil column, and mineralization (CO_2 evolution) during crop cultivation. Thus, a large number of results from outdoor lysimeter experiments have been reported using ^{14}C-labeled chemicals to show their fate in the agroenvironment (Kubiak et al. 1988; Stork et al. 1994; Lee et al. 1994, 2003; Schroll and Kühn 2004; Lee et al. 2005; Schroll et al. 2008). The present investigation is aimed at showing the long-term fate of mefenacet used for the treatment of rice paddy soil using a ^{14}C-radiotracer in a rice-grown lysimeter, simulating the rice paddy conditions over 6 consecutive years.

Materials and methods

Chemical composition of mefenacet

The [aniline-^{14}C]mefenacet used in this experiment was purchased from International Isotopes Inc. (Münich, Germany). As can be seen in Fig. 1, the position labeled with ^{14}C in the chemical structure of mefenacet is the benzene ring carbons of the aniline moiety. The specific radioactivity was 3.4 MBq/mg and the radiochemical purity was greater than 98 %.

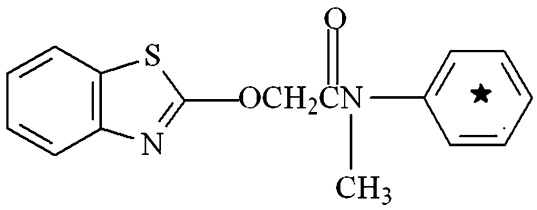

Fig. 1 Structural formula and labeled position (*asterisk*) of mefenacet (2-(1,3-benzothiazol-2-yloxy)-*N*-methylacetanilide)

Lysimeter and preparation of the soil core

The lysimeter used in this investigation was manufactured with stainless steel (8 mm in thickness). The surface area was 0.25 m^2, the height was 1.1 m, and the inner diameter was 0.564 m. The undisturbed soil core was obtained from rice paddy fields by pressing the lysimeter down into a rice paddy located at Bokdae-dong, Cheongju, Korea, with the aid of a fork crane. Table 1 presents the properties of the soil.

Application of [^{14}C]mefenacet and cultivation of rice plants

Before transplanting, the lysimeter soil was fertilized with N–P–K at the ratio of 150–90–110 kg/ha; 80 % of the total nitrogen fertilizer was applied just before transplanting and the rest at the earing stage. Rice seedlings (*Oryza sativa* cv. Akibare, 33–39 days old) were transplanted every year into the lysimeter soil, at the rate of nine hills of three seedlings per hill. [^{14}C]Mefenacet was applied to the lysimeter soil 70 days after transplantation as a mixture of the commercial granular formulation, Mannyang® (3.5 % mefenacet + 0.07 % pyrazosulfuron-ethyl + 96.43 % adjuvant), at the recommended application rate of 1.05 kg a.i. mefenacet/ha in Korea. The commercial granular formulation was macerated to powder in a mortar before mixing to ensure homogeneous application and convenience in mixing with soil used as the diluent. [^{14}C]Mefenacet (10.561 MBq, equivalent to 3.106 mg a.i.) dissolved in 10 mL methanol and 0.657 g (equivalent to 23.00 mg a.i. mefenacet) of the commercial granular formulation were added to about 200 g of soil. After methanol was completely evaporated, the soil was mixed well and scattered over the lysimeter soil surface. The leachates from the lysimeter were collected in a 2 L plastic container, and the volume and radioactivity measured biweekly. Rice plants were grown for 136 days until harvest, according to the

Table 1 Physicochemical properties of the lysimeter soil layers

Soil depth (cm)	pH H_2O (1:5)	OM[a] (%)	P_2O_5 (mg kg)	K (cmol(+)/kg.soil)	Ca (cmol(+)/kg.soil)	Mg (cmol(+)/kg.soil)	C.E.C. (cmol(+)/kg.soil)	SiO_2 (mg/kg)	Sand (%)	Silt (%)	Clay (%)	Texture
0–10	4.6	3.1	107	0.28	3.9	0.9	8.6	38	45.8	42.6	11.6	L[b]
10–20	5.1	2.4	67	0.18	3.6	0.9	6.7	52	46.8	37.4	15.8	L
20–30	6.4	0.8	7	0.16	5.2	1.5	7.5	70	57.4	28.7	13.9	SL[c]
30–40	6.4	1.0	1	0.23	6.0	1.7	8.8	131	34.2	43.5	22.3	L
40–50	6.3	0.8	0	0.23	6.7	2.4	10.2	178	34.8	42.9	22.3	L
50–60	6.2	0.7	1	0.21	7.0	2.8	10.9	230	31.2	43.0	25.8	L
60–70	6.1	0.9	0	0.21	6.7	2.7	10.5	138	31.2	42.5	26.3	L
70–80	6.2	1.1	1	0.18	6.2	2.5	9.7	149	37.8	38.7	23.5	L
80–90	6.1	1.2	5	0.15	6.2	2.4	9.6	161	39.6	38.0	22.4	L
90–100	6.2	0.8	4	0.19	5.5	2.4	9.0	141	42.2	33.0	24.8	L

[a] Organic matter

[b] Loam

[c] Sandy loam

conventional farming methods in Korea. Throughout the cultivation period, the soil was flooded as the rice paddies would be; in Korea, rice crops are usually grown under flooded conditions almost until harvest. Accordingly, during the dry season, tap water was supplied for additional irrigation. To prevent the run-off of [^{14}C]mefenacet from flooded soils by heavy rain, the lysimeter was roofed with vinyl film during the rice-growing period. After harvest each year, the lysimeter was kept open until the following cultivation, and the leachate was collected continuously. A diagram of the lysimeter illustrating the growing of rice plants and the collection of leachate is given in Fig. 2 (Lee et al. 1994).

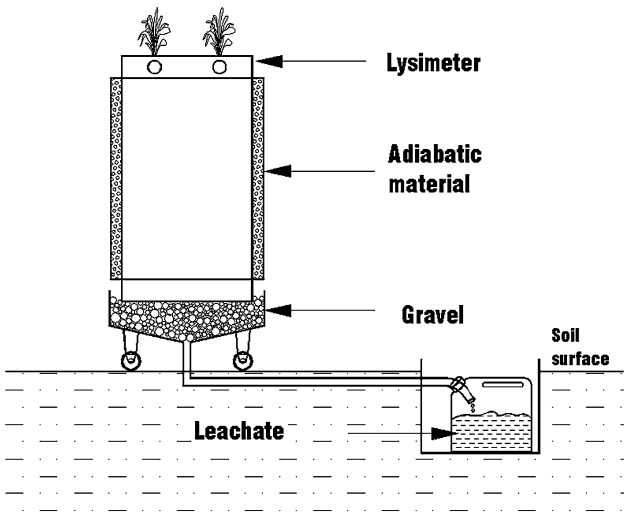

Fig. 2 Diagram of the lysimeter showing the growth of rice plants and the collection of leachate (Lee et al. 1994)

Mineralization and volatilization of mefenacet during rice cultivation

The $^{14}CO_2$ and volatile substances that evolved from the mefenacet-treated soil surface were trapped with a special device made of Pyrex® glass (Lee et al. 1994). Four devices were placed on the surface of the soil, each covered an area of 50.24 cm^2 representing about 1/50 of the lysimeter surface area. The $^{14}CO_2$ and volatile substances were trapped in 1 N NaOH and 0.1 N H_2SO_4, respectively, and the ^{14}C-radioactivity was measured biweekly.

Soil and rice plant sampling

After harvest, a soil sample from each 5 cm layer was collected to a depth of 40 cm in the first year, to 50 cm in the second year, to 45 cm in the fourth year, and to 90 cm in the sixth year using a soil core sampler attached to a stainless steel core of 5.05 cm diameter, 5 cm length, and 100 cm^3 volume. The samples were taken randomly from three spots, and those of each layer were combined, air dried, and ground in a mortar for analysis. The harvested rice plants were separated into straw, ears without rice grain, chaff, and brown rice grain; freeze-dried with a freeze drier (Chem Lab. Instruments LTD., SB4, England); and pulverized with a blender (Philips model HR 2094, China) to measure the radioactivity by combustion. Brown rice grain was obtained by removing chaff.

Extractable and non-extractable ^{14}C-soil residues

Each 150 g of soil sample was collected from 0 to 5 and 5 to 10 cm depths from the lysimeter, and was exhaustively extracted with acetone and methanol consecutively. The combined extracts were then subjected to ^{14}C-radioactivity

measurement. Soils extracted previously with the solvents were combusted to determine the non-extractable bound residues; an additional 5 g of the solvent-extracted soils was extracted with 0.1 M $Na_4P_2O_7$. The combined extracts were divided into fulvic and humic acid by acidification (Lee et al. 1991), and then the ^{14}C-radioactivity incorporated into fulvic and humic acid was measured. The remaining extracted soils were combusted for the ^{14}C-radioactivity in the humin fraction.

Measurement of radioactivity

The radioactivities in the soil and rice plants were measured by combusting 0.3 g of soil and 0.2 g of rice plants that had been freeze-dried and using a biological oxidizer (OX-400, R. J. Harvey Instrument Corp., USA) to give $^{14}CO_2$. The flow rate of oxygen and nitrogen in the biological oxidizer was 300 mL/min. The temperatures of the catalyst zone and combustion zone were 700 and 900 °C, respectively, and the combustion time was 4 min. The $^{14}CO_2$ evolved was absorbed in 15 mL of Carbo Max™ Plus (Lumac LSC B. V., The Netherlands). Radioactivity was measured using a liquid scintillation counter (LSC, PW 4700, Philips) with automatic quench correction. The radioactivities dissolved in organic solvents were measured using 15 mL of Ready-organic (Packard, USA) after the solvents were evaporated and those of $^{14}CO_2$ absorbed in 1 N NaOH and volatile substances absorbed in 0.1 N H_2SO_4 were measured using Aquasol (Du Pont, NEN Research Products, USA) as a cocktail.

Microbial activity as measured by DMSO reduction and the number of microbial colonies in the lysimeter soil

The dimethyl sulfoxide (DMSO) reduction rate as a parameter of microbial activity in soil was measured by the method of Alef and Kleiner (1989). To count the number of microorganisms present in the soil, 1 g of soil was added to 10 mL of sterile distilled water and shaken for 2 h. The soil suspension was diluted adequately, 0.1 mL of which was spread on the nutrient broth medium (5 g peptone, 3 g beef extract, and 10 g agar in 1 L distilled water) and incubated at 30 °C for 48 h. The colonies were counted with a colony counter (Seeley et al. 1991).

Results and discussion

Mineralization and volatilization during the experimental period

Figure 3 shows that the total amount of $^{14}CO_2$ that evolved during the experimental period was 12 % of the originally applied ^{14}C-radioactivity. After rice harvest, the soil was maintained under non-flooded dry conditions. However, the $^{14}CO_2$ evolution did not increase with time. Meanwhile, volatilization increased to 0.017 % under dry conditions following harvest. In contrast, in our previous investigations of the insecticide imidacloprid (Lee et al. 2003) and the herbicide azimsulfuron (Lee et al. 2005), $^{14}CO_2$ evolution increased drastically under non-flooded dry conditions following harvest. These results might be due to active microbial and photolytic degradation under these conditions.

Distribution of the ^{14}C in rice plants

As shown in Table 2, the total amount of ^{14}C in the plants was only 1.978 % of the originally applied ^{14}C-radioactivity after harvest in the first year, most of it being detected in straw (1.855 %). The amount measured in brown rice grains was 0.037 % of the originally applied ^{14}C. When this amount was calculated as the concentration of mefenacet equivalent, it corresponds to 0.089 μg/g. In the second year, the amount absorbed and translocated to the various parts of rice plants was 8.7 times lower than that in the first year. In subsequent years, the amount continued to decrease gradually. Krauskopf et al. (1989) reported that mefenacet was rapidly absorbed by leaves of rice and *Echinochloa crus-galli,* whereas root uptake was very slow, especially in rice. They also reported that in soil, mefenacet is strongly adsorbed and less than 10 % was found to be available to rice and *Echinochloa*. In this long-term experiment, the total ^{14}C-radioactivity absorbed and translocated over 6 consecutive years was only 2.46 %. This amount may be small because mefenacet-bound residues remaining in soil were not readily available to rice plants transplanted and grown in the following years.

Leaching

Table 3 presents the total amount of leachate and ^{14}C-radioactivity exuded from the lysimeter soil during the experimental period. As seen in this table, only 0.778 % of the originally applied ^{14}C-radioactivity leached through the soil layer over 6 consecutive years. Previously, Schmidt et al. (1984) suggested low soil mobility of mefenacet. They showed that in a soil thick-layer chromatography study, despite following an application of water equivalent to 27 cm of precipitation over a 3–4-h period, the majority of herbicidal activity remained in the upper 5 cm of soil. In our long-term leaching experiment, which simulated the undisturbed soil layer for 6 years, it was confirmed that mefenacet has very low soil mobility.

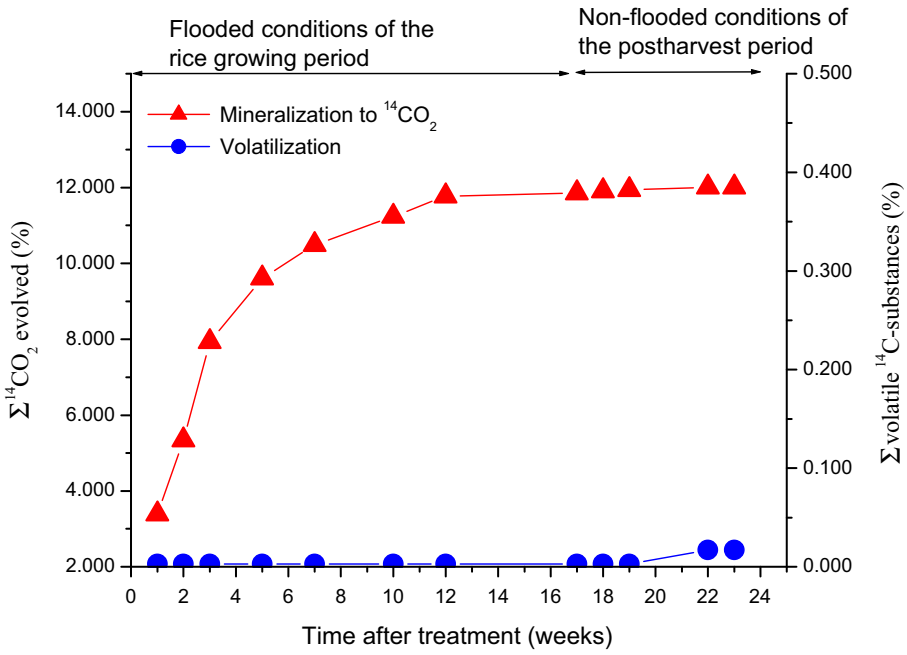

Fig. 3 Mineralization of [^{14}C]mefenacet to $^{14}CO_2$ and volatilization during the lysimeter experiment. ^{14}C-Radioactivity applied = 100 %. 1st–17th week, flooded rice-growing period; 18th–23rd week, non-flooded dry period after harvest

Table 2 Amounts of the ^{14}C-radioactivity absorbed and translocated in each part of rice plants grown in the lysimeter soil treated with [^{14}C]mefenacet. ^{14}C-Radioactivity applied = 100 %

Part of rice plants	^{14}C-Radioactivity (%)						
	1st year	2nd year	3rd year	4th year	5th year	6th year	Total
Straw	1.855 ± 0.047[a] (1.418 ± 0.036)[b]	0.188 ± 0.009 (0.175 ± 0.008)	0.097 ± 0.011 (0.009 ± 0.010)	0.078 ± 0.002	0.020 ± 0.008	0.016 ± 0.000	2.254
Ears without rice grain	0.006 ± 0.000 (0.234 ± 0.007)	0.004 ± 0.000 (0.081 ± 0.050)	0.001 ± 0.010 (0.006 ± 0.000)	0.002 ± 0.001	0.001 ± 0.004	0.002 ± 0.000	0.016
Chaff	0.080 ± 0.004 (0.366 ± 0.017)	0.009 ± 0.001 (0.037 ± 0.002)	0.002 ± 0.004 (0.005 ± 0.000)	0.007 ± 0.001	0.003 ± 0.004	0.002 ± 0.000	0.103
Brown rice grain	0.037 ± 0.006 (0.089 ± 0.014)	0.02 6 ± 0.006 (0.025 ± 0.016)	0.00 3 ± 0.000 (0.004 ± 0.000)	0.006 ± 0.002	0.010 ± 0.001	0.006 ± 0.000	0.088
Total	1.978	0.227	0.103	0.093	0.034	0.026	2.461

[a] Mean ± standard deviation of triplicates

[b] Numbers in parentheses are concentrations of mefenacet equivalent (μg/g) calculated on the basis of the specific ^{14}C-radioactivity (3.4 MBq/mg) of mefenacet applied. After the 4th year, the concentration (μg/g) was too low to be detected

Distribution of the ^{14}C in soil layers

Table 4 presents the distribution of ^{14}C in each lysimeter soil layer after long-term leaching. In the first year, the soil samples were taken down to a depth of 40 cm. The total amount of ^{14}C-radioactivity remaining in the lysimeter soil layers was 82.24 % of the originally applied ^{14}C-radioactivity. Considering the fact that the total amount of $^{14}CO_2$ that evolved during the first 23 weeks was 12 % of the originally applied ^{14}C-radioactivity, much more $^{14}CO_2$ must have evolved during the rest of the experimental period. Because the ^{14}C-radioactivity in the leachate collected in the first year was only 0.001 % (Table 3) and the ^{14}C absorbed and translocated by rice plants was 1.978 % (Table 2), the rest of the ^{14}C-radioactivity must have been lost by mineralization to $^{14}CO_2$. Even if the loss by volatilization was negligible, it would have increased during the non-flooded dry period following harvest (Fig. 3). In the second year, the soil samples were taken down to 50 cm. In this case, the total amount of the ^{14}C

Table 3 Amounts of ^{14}C-radioactivity leached through the lysimeter soil treated with [^{14}C]mefenacet for 6 consecutive years. ^{14}C-Radioactivity applied = 100 %

Period (year)	Volume of leachate (L)	^{14}C-Radioactivity leached (%)	Original amount applied (mg)	Amount leached (mg)
1st	20.510	0.001	26.106	0.0003
1st–2nd	44.979	0.011	26.106	0.003
1st–3rd	112.609	0.054	26.106	0.014
1st–4th	230.209	0.149	26.106	0.039
1st–5th	358.289	0.314	26.106	0.082
1st–6th	473.069	0.778	26.106	0.203

Table 4 Amounts of ^{14}C-radioactivity remaining at different depths of the lysimeter soils treated with [aniline-^{14}C]mefenacet after harvest. ^{14}C-Radioactivity applied = 100 %

Soil depth (cm)	^{14}C-Radioactivity (%)					
	1st	2nd	3rd	4th	5th	6th
0–5	64.74	34.17	No sampling[a]	17.31	No sampling[a]	14.63
5–10	14.41	11.18		15.96		15.34
10–15	1.15	1.76		13.07		9.24
15–20	0.60	0.72		1.66		1.56
20–25	0.49	0.56		0.41		0.84
25–30	0.39	0.57		0.33		0.54
30–35	0.29	0.44		0.24		0.42
35–40	0.17	0.32		0.06		0.29
40–45		0.16		0.06		0.33
45–50		0.16				0.21
50–55						0.39
55–60						0.19
60–65						0.10
65–70						0.09
70–75						0.15
75–80						0.11
80–85						0.05
85–90						0.10
Total	82.24	50.04		49.1		44.58

[a] To minimize disturbance of the lysimeter soil profile, sampling was done biennially beginning the second year

remaining in the soil layers was 50.04 %. The ^{14}C remaining in the 0–5-cm layer was 67.74 % in the first year and 34.17 % in the second year. This means that mefenacet moved downward slowly but steadily. The gradual decrease in the ^{14}C in the soil layers indicates that more $^{14}CO_2$ might have evolved during the period, even if it was not observed in this study. The ^{14}C detected in the leachate collected until the second year was only 0.011 % of the originally applied ^{14}C (Table 3). This indicates that very small amounts of ^{14}C are lost by leaching. To minimize the disturbance of the lysimeter soil profile, sampling was done biennially beginning the second year. In the fourth year, the soil samples were taken down to 45 cm. The total amount of the ^{14}C remaining in the soil layers was only 49.1 % of the originally applied ^{14}C; almost half of the ^{14}C was therefore lost. In the final year, the soil samples were taken down to 90 cm. The total ^{14}C remaining in the soil layers was 44.58 %; in these samples, more than half of the ^{14}C-radioactivity applied was lost. In the sixth year, the ^{14}C-radioactivity remaining in the 0–5-cm layer was only 14.63 %. This is 4.4 times lower than the ^{14}C remaining in the same layer in the first year (64.74 % of the originally applied ^{14}C). This trend indicates that even if most of the ^{14}C-radioactivity was lost, ^{14}C moved downward slowly.

Formation of bound residues and change in the polarity of ^{14}C in soil extracts

The formation of non-extractable bound residues was examined 136 days following [^{14}C]mefenacet treatment. Based on our previous investigations (Lee et al. 1991, 1988), the period of 136 days was sufficiently long for the

Table 5 Formation of non-extractable bound residues of [^{14}C]mefenacet in the lysimeter soil[a]

Soil depth (cm)	^{14}C Extracted (%)			Non-extractable bound residue (%)
	Acetone	Methanol[b]	Total	
0–5	3.32	1.69	5.01	94.99
5–10	2.16	1.30	3.46	96.54

[a] Examined 136 days after the [^{14}C]mefenacet treatment

[b] Acetone extraction was followed by methanol extraction

formation of non-extractable bound residues of mefenacet. As shown in Table 5, about 95–97 % of the applied [^{14}C]mefenacet was strongly adsorbed in the upper 0–10-cm layer to form non-extractable bound residues. This soil layer (0–10 cm) contained 3.1 % organic matter, which is a higher proportion than is present in the deeper layers (Table 1). Nakamura et al. (1996) investigated the influence of organic matter content on adsorption of mefenacet to soil and the phytotoxic activity of mefenacet in relation to its concentration in soil water varying in organic matter content. They demonstrated that the phytotoxic activity of mefenacet applied to soils containing various amounts of organic matter decreased as the organic carbon content increased, and the adsorption in the solid phase decreased with a decrease in organic carbon content. In this study, the ^{14}C extracted with acetone followed by methanol was only 5.01 and 3.46 % from the 0–5 and 5–10-cm layers, respectively. The differences in the amount extracted could be due to the differences in the organic carbon content, even if it was not differentiated between the 0–5 and 5–10-cm layers in our soil analysis. In the soil-bound residues of [^{14}C]mefenacet, more than half of the non-extractable bound residues were characterized in the humin fraction (52–66 %), which was essentially inactive biologically 136 days after [^{14}C]mefenacet treatment (Table 6; Nam and Kim, 2002).

Microorganisms in soil as affected by rice cultivation

Alef and Kleiner (1989) presented a method for the determination of microbial activities in soils and soil aggregates, based on the enzymatic reduction of DMSO to dimethyl sulfide (DMS). Using their method, in this study, the number of microbial colonies in the topsoil (0–10-cm depth) and their activity were observed to have increased greatly after rice cultivation (Table 7); the number of colonies was about 45 times higher and the microbial activity was about 1.4 times higher following rice cultivation. This could be ascribed to the fact that various kinds of exudates accumulated in the rhizosphere (Rovira 1965; Kimura et al. 1977; Curl 1986). A review by Rovira (1965) shows the wide array of substances that have been found in root exudates, including carbohydrates, amino acids, organic acids, and enzymes. Kimura et al. (1977) also found that various kinds of sugars, amino acids, and organic acids had accumulated in the rhizosphere of rice plants. Microorganisms in the rhizosphere could use these substances as their nutrients.

Fate of [^{14}C]mefenacet in a rice plant-grown lysimeter

Table 8 summarizes the fate of [^{14}C]mefenacet used as a one-time treatment on the lysimeter soil containing transplanted rice plants and grown every year for 6 consecutive years. In the first year, the recovery accounted for 96.2 % of the originally applied ^{14}C-radioactivity. As time went on, the recovery dropped sharply, and was 64.3 % in the second year. This result indicates that most of the [^{14}C]mefenacet residue in soil was lost by the evolution of $^{14}CO_2$ during the second year. This finding is supported by the fact that after the harvest of rice plants in the first year, most of the residues of [^{14}C]mefenacet and its degradation

Table 6 Distribution of the non-extractable [^{14}C]soil-bound residues in humic substances of the lysimeter soil

Soil depth (cm)	Non-extractable bound residues[a)] (%)	Humic substances		
		Fulvic acid (%)	Humic acid (%)	Humin (%)
0–5	94.99	18.40	29.36	52.24
5–10	96.54	18.54	15.79	65.67

Fulvic acid + humic acid + humin = 100 %

[a] 136 days after [^{14}C]mefenacet treatment

Table 7 Comparison of the number of microbial colonies and the microbial activities as indicated by the DMSO reduction in the topsoil (0–10-cm depth) of the lysimeter soil before and after the cultivation of rice plants[a]

Cultivation of rice plants	Number of colonies[b] ($\times 10^5$ CFU[c]/g soil)[d]	DMS formed[b, e] (ng DMS/g dry weight soil/h)
Before	3.53 ± 0.12	97.37 ± 1.12
After	157.06 ± 3.26	138.81 ± 1.53

[a] Done in the first year

[b] Mean ± standard deviation of triplicates

[c] Colony-forming unit

[d] Dry weight basis

[e] The DMSO reduction rate is expressed as nanograms. DMS per gram dry weight per hour

Table 8 Fate of [^{14}C]mefenacet after treatment onto rice plant-grown lysimeter soil during the experimental period of 6 consecutive years. ^{14}C-Radioactivity applied = 100 %

Period (year)	% of applied ^{14}C					
	$^{14}CO_2$ evolved[a)]	^{14}C volatilized[a]	^{14}C in rice plants (except for root)	^{14}C leached	^{14}C in soil	Recovery
1st	12	0.017	1.978	0.001	82.24[c]	96.236
1st–2nd	12 + α[b]	0.017 + α[b]	2.205	0.011	50.04[d]	64.273
1st–3rd	12 + α[b]	0.017 + α[b]	2.308	0.054	–[e]	–[h]
1st–4th	12 + α[b]	0.017 + α[b]	2.401	0.149	49.1[f]	63.667
1st–5th	12 + α[b]	0.017 + α[b]	2.435	0.314	–[e]	–[h]
1st–6th	12 + α[b]	0.017 + α[b]	2.461	0.778	44.58 [g]	59.836

[a] ^{14}C mineralized and volatilized during a 23 week period within the first year of application of [^{14}C]mefenacet

[b] Loss by $^{14}CO_2$ evolution and volatilization not measured from the second to the sixth year

[c] 1Down to 0–40-cm soil layer

[d] Down to 0–50-cm soil layer

[e] No sampling in this year

[f] Down to 0–45-cm soil layer

[g] Down to 0–90-cm soil layer

[h] Recovery was not calculated because ^{14}C-radioactivity was not measured in each soil layer of the lysimeter

products (64.74 %) remained in the top 0–5-cm of soil. These residues on the surface of soil are vulnerable to aerobic microbial degradation, photolysis, and many other environmental factors. It is important to note that the lysimeter soil was totally exposed to the natural conditions from October through May of the following year, until transplantation of the rice plants in the following season. Accordingly, this period is not flooded so that conditions are not suitable for microbial degradation and other dissipating factors. Even if the ^{14}C in rice plant roots could be measured, the possibility of finding a significant amount of the ^{14}C-radioactivity would be very low, because uptake of mefenacet by rice roots has been reported to be very slow (Krauskopf et al. 1989). Furthermore, this argument is based on the fact that only 1.978 % of the originally applied [^{14}C]mefenacet was absorbed and translocated into the various parts of the rice plants in the first year. The total amount of the ^{14}C-radioactivity leached over the 6-year period was only 0.778 %, indicating that the downward movement of mefenacet in soil is very slow, but steady. In conclusion, the strong adsorption of [^{14}C]mefenacet onto soil organic matter, the formation of large amounts of non-extractable bound residues in the upper soil layer (0–10 cm), and the low detection of the chemical in rice grains observed in this study indicate that exposure of groundwater and crops to [^{14}C]mefenacet does not necessarily pose a health risk.

Acknowledgments We are grateful to the Korea Science and Engineering Foundation for financial support.

References

Abbott CK, Sims RC, Sorensen DL (1992) Use and efficiency of ethylene glycol monomethyl ester and monoethanolamine to trap volatilized [7-^{14}C]naphthalene and $^{14}CO_2$. Environ Toxicol Chem 11:181–185

Alef K, Kleiner D (1989) Rapid and sensitive determination of microbial activity in soils and in soil aggregates by dimethyl sulfoxide reduction. Biol Fertil Soils 8:349–355

Curl EA, Truelove B (1986) Root exudates (chapter 3). The rhizosphere. Springer, New York, pp 55–92

Fedtke C (1991) Mode of action studies with mefenacet. Pestic Sci 33:421–426

Führ F, Mittelstaedt W (1982) Influence of experimental and certain environmental factors on the uptake of soil-applied herbicides. In: Proceedings of the 5th IUPAC Congress Pesticide Chemistry: human welfare and the environment, Vol. 4. Pergamon Press, Oxford, pp 183–188

Hess FD, Holmsen JD, Fedtke C (1990) The influence of the herbicide mefenacet on cell division and cell enlargement in plants. Weed Res 30:21–27

Ito S, Kamochi A, Sawada K, Goto T, Yasui K (1989) Mefenacet, a new paddy herbicide(3). In: Proceedings, 12th APWSS Conference, Seoul, pp 255–260

Ito S, Ueno C, Goto T, Yasui K (2000) Morphological and anatomical effects of mefenacet on the growth of barnyard grass (*Echinochloa oryzicola* Vasing.). J Weed Sci Technol 45:81–87

Kimura M, Wada H, Takai Y (1977) Studies on the rhizosphere of paddy rice (part 4): physical and chemical features of rhizosphere (II). J Sci Soil Manure 48:540–545

Krauskopf B, Fedtke C, Feucht D (1989) Uptake, translocation, and metabolism of mefenacet in rice and *Echinochloa*, and the mechanism of the synergistic interaction with edifenphos. In: Proceedings 4th EWRS Mediterranean Symposium, pp 386–393

Kubiak R, Führ F, Mittelstaedt W, Hansper M, Steffens W (1988) Transferability of lysimeter results to actual field situations. Weed Sci 36:514–518

Lee JK, Führ F, Mittelstaedt W (1988) Formation and bioavailability of bentazon residues in a German and Korean agricultural soil. Chemosphere 17:441–450

Lee JK, Kyung KS, Führ F (1989) Bioavailability of soil-aged residues of the herbicide bentazon to rice plants. J Korean Soc Appl Biol Chem 32:393–400

Lee JK, Kyung KS, Wheeler WB (1991) Rice plant uptake of fresh and aged residues of carbofuran from soil. J Agric Food Chem 39:588–593

Lee JK, Führ F, Kyung KS (1994) Behaviour of carbofuran in a rice plant-grown lysimeter throughout four growing seasons. Chemosphere 29:747–758

Lee JK, Führ, F, Ahn KC, Kwon JW, Park JH, Kyung KS (2003) Fate of the insecticide imidacloprid treated to the rice-grown lysimeters during three consecutive cultivation years. ACS symposium series 853

Lee JK, Führ F, Park SH, Lee EY, Kim YJ, Kyung KS (2005) Long-term fate of the herbicide azimsulfuron in a rice-grown lysimeter over six consecutive years. ACS symposium series 899

Nakamura N, Kobayashi K, Shim IS, Nagatsuka S (1996) Influence of soil organic matter content on mefenacet concentration in soil water and phytotoxic activity. Weed Res 41:339–343

Nam K, Kim JY (2002) Role of loosely bound humic substances and humin in the bioavailability of phenanthrene aged in soil. Environ Pollut 118:427–433

Rovira AD (1965) Plant root exudates and their influence upon soil microorganisms. In: Baker KF, Snyder WC (eds) Ecology of soil-borne plant pathogens-prelude to biological control. University of California, California, pp 413–414

Scheunert LD, Vockel D, Schmitzer J, Viswanathan R, Klein W, Korte F (1983) Fate of chemicals in plant-soil systems: comparison of laboratory test data with results of open air long-term experiments. Ecotoxicol Environ Saf 7:390–399

Schmidt RR, Eue L, Förster H, Mues V, Aya M (1984) Mefenacet—a new herbicide. Mededе van de Fac Landbouwwet Rijksuniv Gent 49(3b):1075–1084

Schroll R, Kühn S (2004) Test system to establish mass balances for ^{14}C-labeled substances in soil-plant-atmosphere systems under field conditions. Environ Sci Technol 38:1537–1544

Schroll R, Grundmann S, Dörfler U, Ruth B, Munch JC (2008) Lysimeter experiments to investigate the fate of chemicals in soil-comparison of five different lysimeter systems. Water Air Soil Pollut 8:209–216

Seeley HW Jr, Van Demark PJ, Lee JJ (1991) Microbes in action, 4th edn. W. H. Freeman and Company, New York

Stork A, Witte R, Führ F (1994) A wind tunnel for measuring the gaseous losses of environmental chemicals from the soil/plant system under field-like conditions. Environ Sci Pollut Res 1:234–245